Review

CARDIOPULMONARY METASTRONGYLOIDOSIS OF DOGS AND CATS CONTRIBUTION TO DIAGNOSE

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Abstract

Background. In the last fifteen years on the European continent and also worldwide, the prevalence of cardiopulmonary metastrongyloidosis in dogs and cats has increased significantly, especially cases involving those parasites which are the most important for veterinary practice (*Angiostrongylus vasorum*, *Aelurostrongylus abstrusus* and *Crenosoma vulpis*).

Scope and Approach. The aim of this study is to present a detailed clinical-parasitological approach to highlight the importance of these helminths, and to display the newest findings concerning the diagnostic possibilities in dogs and cats

Key Findings and Conclusions. The effects of global warming, vector range shift, the frequent transportation and movement of animals to other epizootic areas, as well as the intensification of merchandise transportation and movement of people are just some of the potential factors which could impact the dynamics of incidence, upkeep and spread of cardiopulmonary nematodoses in carnivores. For the timely implementation of effective treatment of sick animals, it essential to accurately diagnose these parasitoses. Accurate, timely diagnosis can, in the end, significantly contribute to the prognostic course of disease in infected carnivores. Cardiopulmonary metastrongyloidoses in dogs and cats have great clinical-parasitological significance because of their high degree of pathogenicity, their spread outside endemic areas, the difficulties encountered in establishing their diagnosis, and the fact that they represent a potential danger to human health.

Key Words: angiostrongylosis, aelurostrongylosis, crenosomosis, dog, cat, diagnostics.

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INTRODUCTION

Cardiopulmonary metastrongyloids of dogs and cats belong to the phylum: Nemathelminthes, class: Nematoda, subclass: Secernentea, order: Strongylida, superfamily: Metrastrongyloidea and families: Angiostrongylidae and Crenosomatidae (Anderson, 1978). For veterinary practice, the most important species are: *Angiostrongylus vasorum*, *Aelurostrongylus abstrusus* and *Crenosoma vulpis*. These nematodes have indirect life cycles, which demand the presence of intermediate hosts (biological vectors), and part of their development is in gastropods. The possibility of dog and cat infection is not conditioned only by the presence of vectors, but it is proven that infection of dogs and cats also depends on the level and number of causes (Mandić, 2015).

The interest of scientific and professional public in these parasitoses has increased from the moment when the causal factors spread outside the endemic area. Ecological changes have occurred as a result of global warming, and they have significantly influenced the population growth of wild carnivores (Ilić et al., 2016a), as well as the density of feral dogs and cats, in city and suburban areas (Đurić et al., 2011; Lažetić et al., 2012). This resulted in significant increases in populations of possible reservoirs of infection for pets, which created the preconditions for the constant presence and expansion of zoonotic parasites (Obrenović et al., 2003; Nikolić et al., 2008; Ilić et al., 2017), including cardiopulmonary parasites.

A. vasorum is the causative agent of clinical angiostrongylosis in dogs, and its popular name is "French heartworm", because the first time it was diagnosed as the causative agent of an endemic disease was in France in the 19th century (Morgan et al., 2005; Jefferies et al., 2010). This worm parasitises the lung tissue and the right heart of canids. Infection can occur in dogs of all ages, with clinically manifested cardiopulmonary ailments, neurological symptoms, problems with coagulation and signs of hypertension and generalized disease. Hypertension occurs as an accessory symptom in the course of disease conditions, and blood pressure can depend on the renin-angiotensin system, aldosterone, prostaglandin, the adrenergic system, and age, sex, race, temperament, environment, number of angiostrongylids present and, in part, also how and where the pressure measurement was taken (Stepanović and Nikolovski-Stefanović, 2005). However, the greatest number of clinical cases has been recorded in dogs younger than two years old (Chapman et al., 2004; Koch and Willesen, 2009). *Angiostrongylus* species that parasitise dogs and rats are also important, such as: *A. cantonensis*, and *A. costaricensis*, which parasitises only rats (Spratt, 2015). This infection primarily produces an inflammation with eosinophilia, but in dogs and rats, some changes in acute phase proteins can also be found: concentration increases (fibrinogen, haptoglobin, α-2 macroglobulin, C-reactive protein, complement factors, monozin binding protein), and; negative in the case of concentration decreases (albumin, transferin, retinol binding protein) (Stepanović et al., 2011). Thus, it was supposed that the presence of parasites produces an acute phase response (Stepanović et al., 2011).

Angiostrongylus cause established zoonoses, manifesting in neurological and abdominal angiostrongylosis. People are accidental hosts and can be infected by third stadium larvae contaminating food or water; this leads to meningitis, eosinophilia and intestinal granulomas (Wang et al., 2008; Helm et al., 2010; Spratt, 2015).

Aelurostrongylosis is caused by *A. abstrusus,* which parasitises the bronchioles and lung tissue of cats (Traversa et al., 2010; Mircean et al., 2010; Di Cesare et al., 2011; Knaus et al., 2011; Ramos et al., 2012; Barutzki and Schaper, 2009; Capári et al., 2013; Riggio et al., 2013; Spada et al., 2013; Waap et al., 2014) and other felines, most often in the Siberian leopard cat (*Felis bengalensis euptilurus*) (Gonzáles et al., 2007) and Eurasian lynx (*Lynx lynx*) (Szczesna et al., 2006). The pathological importance of the agent is expressed in infections of high intensity, when the parasites cause bronchopneumonia in cats (Lalošević et al., 2001).

Since cats are hunting paratenic host and transitional hosts, they are at risk of *A. abstrusus* infection. It is reasonable to suspect that this helminthosis is widespread in cats in Serbia, but not enough explored. It is likely that in many cases of the infection, the aetiological agent remains undiagnosed, or the symptoms are misinterpreted. Therefore, a diagnostic review of the cardiopulmonary metastrongylids in cats could contribute to adequate selection of treatment methods in suspected cases of pneumonia, as well as determining the current prevalence of aelurostrongylosis.

The lung nematode *C. vulpis* is the predominant causal agent of respiratory infections in foxes in North America and Europe, and sporadic cases have been recorded in dogs. Because of the clinical variations and the fact that the clinical signs can be unspecific, it is hard to produce a correct diagnosis, so crenosomosis can be an important health issue for veterinary medicine (Rinaldi et al., 2007). By parasitising the trachea, bronchi and bronchioles of feral and domestic canids, the adult forms scar the parenchyma in hosts' lungs and cause chronic bronchitis, followed by sneezing, wheezing and chronic dry or mucosal coughing. Infections of high intensity can cause death by bronchopneumonia and respiratory insufficiency (Holmes and Kelly, 1973; Bihr and Conboy, 1999; Bowman et al., 2002; Taylor et al., 2007). The cohabitation of foxes with feral dog and cat populations can be a potential source of transfer of *C. vulpis* and other pulmonary nematodes in urban areas, with which the risk gets higher for zoonotic transfer of the parasite (Simin et al., 2012; Ilić et al., 2016b).

The lack of specific clinical symptoms and the difficulties in differential diagnostics can lead to delays of proper, or even incorrect, diagnosis. This is why it is important that in pets displaying cardiological and respiratory disorders, the presence of cardiopulmonal metastrongyloidosis should not be excluded without investigation.

ANGIOSTRONGYLOSIS DIAGNOSTICS

The precise diagnosis of canine angiostrongylosis can be established based on the clinical signs, with the use of imaging techniques (radiography, echo-cardiography,

computer tomography, MRI and myelography), by examination of blood and cerebrospinal fluids, parasitological section and coprological examination.

Clinical examination

With lung auscultation, the examiner usually receives normal values, with the appearance of increased vesicular breathing. In chronic pulmonary hypertension, caused by pulmonary artery thrombosis resulting from parasite larvae, heart noises can be heard in the area of the tricuspid valve (Stepanović and Nikolovski-Stefanović, 2005; Traversa and Guglielmini, 2008). The localization of the changes can differ due to parasite movement in the heart (Nicolle et al., 2006). Some published studies of clinical signs presented by dogs were due to the typical pathogenic mechanisms caused by *A. vasorum*, i.e. inflammation triggered by parasite eggs and larval stages in the lungs, and by damage caused by adult worms in the pulmonary vessels (reviewed in Morgan et al., 2010). The respiratory signs included cough, haemoptysis and pulmonary oedema. In the heart, signs of congestive heart failure were seen, with marked mitral murmur, Reports of fatal haemorrhage due to rupture of the femoral artery were caused by the adult parasite in an abnormal location (Di Cesare et al., 2015). In particular, infected dogs suffer from obstructed thrombotic endarteritis and fibrosis, and additionally, the parasite induces alterations of metabolic pathways (e.g. chronic Disseminated Intravascular Coagulation, DIC) (Morgan and Shaw, 2010; Gallagher et al., 2012).

Diagnosis with imaging techniques

Imaging techniques can provide useful diagnostic data, especially in the respiratory and neurological cases. In patients which express neurological symptoms, it is very desirable during diagnosis to use MRI and myelography (Wessmann et al., 2006). MRI is used to localize and make visible the lesions in the central nervous system, and for now, it is the most trusted method for detecting the degree of intracranial and intramedular damage. Although it is theoretically possible to detect the parasites, there is little chance that this actually happens in practice, and so far there have been no cases of visualizing the parasites (Whitley et al., 2005). Therefore, we should consider some kind of contrast shooting to improve diagnostics.

Radiography of the chest cavity usually uncovers multifocal changes, which are localized in the interstitial tissue of the bronchi and alveoli, especially on the periphery of the lung (Willesen et al., 2009). As the disease progresses, these changes will spread to the whole of the lungs, which is associated with the formation of granulomas, and haemorrhages (Helm et al., 2010). In severe chronic conditions, the shadows of interstitial lung cancer can be seen, which arise as a result of consolidation and pulmonary fibrosis. After reparation, interstitial lung shadows are slightly visible. Dilatation of the right side of the heart can be noticed, and dilatation of the pulmonary truncus and the blood vessels in the basin of the pulmonary veins are strongly overcharged, which in advanced stages can lead to pulmonary hypertension.

Haemothorax and mediastinal expansion can occur (Boag et al., 2004; Traversa and Gugleilmini, 2008).

High-resolution computed tomography can accurately examine the lesions that occur in the lungs. Consolidation of the lungs can be noticed, especially in the peripheral part of the lobe, as can unevenly distributed multifocal shadows. In severe cases, there may be a diffuse shading deployed along the entire lung, due to infiltration of the lung tissue with blood cells (Koch and Willesen, 2009; Helm et al., 2010).

Echo and Doppler examination of the heart are standard methods for determining morphological changes and heart function. During these tests, enlargement of the right atrium and ventricle was observed (with a consequent reduction in the left ventricular pressure and changes in the pulmonary artery and pulmonary circulation), as well as secondary tricuspid regurgitation in valves (Nicolle et al., 2006). However, all these described changes are not always present, and in any case, they are not pathognomonic for angiostrongylosis.

Blood and cerebrospinal fluid analyses

Blood tests indicate the most important changes: regenerative anaemia, eosinophilia, thrombocytopaenia, leucocytosis, and rarely, neutrophilia (Chapman et al., 2004; Willesen et al., 2009). Changes are not registered in the concentrations of ALT, GGT, urea or creatinine, but there is an increase in α-, β- and γ-globulins during the acute phase (Cury et al., 2005). AST is increased slightly, together with the creatine kinase isoenzyme. This enzyme is an indicator of heart damage and its level increases in parallel with the arrival of parasites in the heart, and the emergence of the initial lesion (Cury et al., 2005). In cases with bleeding, it is necessary to pay attention to the extended clotting time and increased von Willebrand factor (Whitley et al., 2005). Prothrombin time and activated partial thromboplastin time, and also D-dimer levels may be elevated, while fibrinogen is decreased (Ramsey et al., 1996). In animals exhibiting neurological clinical symptoms, it is necessary to examine the cerebrospinal fluid. The parameters typical of nervous stages of angiostrongylosis in dogs are: high protein, signs of erythrophagia and increased number of red blood cells, while the white blood cell count remains within the physiological norms (Wessman et al., 2006).

Parasitological dissection

During parasitological dissection there are no pathognomical changes apparent. On the lungs, granulomatous pneumonia can be observed, with purulent inflammation and eosinophilic infiltration of the changes of the blood vessels in the form of thrombosis and fibrosis. Adult forms of the parasite are localised in the arteries and in the right heart and are surrounded by fibrin. The larvae are found in the tiny blood vessels of the lungs where they cause inflammation, which causes caseous granulomatosis on the peripheral parts of the lung and the affected pleura (Bourque et al., 2008; Denk et al., 2009; Koch and Willesen, 2009). Through the bloodstream, the larvae can scatter to

the brain, kidneys, spleen, adrenal and tracheobronchial lymph nodes, where they can form caseous granulomas (Bourque et al., 2008). Larvae can become localized in the eye, pericardium, pancreas, liver, muscles or skin (Perry et al., 1991; Oliveira-Júnior et al., 2004). Myocarditis or glomerulonephritis can occur, which can lead to death in dogs (Gould and McInnes, 1999). In cases of bleeding, large haematomas may occur, and if there were obvious neurological symptoms, bleeding can be noticed in the brain and spinal cord (Garosi et al., 2005; Bourque et al., 2008).

Coprological examination

First stage larvae (L1) of *A. vasorum* can be diagnosed in faeces; they are 334-380 μm in length and are identified by the appearance of the final body parts (tails), which have a distinctive tip and notch. These larvae have typical cuticles with a serrated dorsal side. There is also a small ventral indentation (Deplazes, 2006; Bourque et al., 2008; McGary and Morgan, 2009).

Faecal samples can be examined by making a faecal smear with the method according to Baermann. In urgent cases, direct faecal smear is used, which has a 54-61% test sensitivity (Humm and Adamantos, 2010). The method according to Baermann is the standard procedure for diagnosing angiostrongylosis. Its drawback is that fresh faecal samples must be used. Some authors have reported negative results for coprological examination using Baermann`s technique in dogs suffering from angiostrongylosis (Oliveira-Júnior et al., 2006; Denk et al., 2009). During the prepatent period, which lasts quite a long time (38-108 days), the larvae cannot be observed in the faeces, regardless of what symptoms the animals exhibit. Negative findings using Baermann`s technique do not exclude the existence of *A. vasorum* nematode infection in an animal, especially when there are clinical signs and the animal is from a risky area (Traversa and Guglielmini, 2008; Helm et al., 2010).

During differential diagnosis, the larvae of *A. vasorum* can be replaced as the larvae of *Crenosoma vulpis* or free wild larvae (McGary and Morgan, 2009). Contamination with free wild larvae can be avoided by reviewing fresh faeces samples, taken directly from the rectum. Collecting daily samples of faeces will increase the sensitivity of Baermann's technique, since the secretion of larvae during the day can vary and they might remain unnoticed if only one sample is tested (Denk et al., 2009).

Bronchoalveolar lavage

In cases where it is not possible to diagnose with Baermann`s method and respiratory symptoms are present, the bronchoalveolar lavage technique is used. In cases with lesions on the lungs, the findings can be determined by an increased number of eosinophils, neutrophils, polynuclear giant cells, and parasites (Barçante et al., 2008). The disadvantages of this technique are the potential risks and the possibility of death due to suffocation of the patient, the need for sedation, the small amount of sample that is obtained, and the fact that this test will be negative unless there are significant lesions in the lungs (Chapman et al., 2004).

Serological and molecular techniques

In serological diagnostic methods, problems can occur due to cross reactivity with antigens of various parasites, and inability to distinguish old and emerging infections. New tests are under development and are largely diagnostically promising, but not yet available on the market (Al-Sabi et al., 2010). Verzberger-Epstein et al. (2008) have synthesized a sandwich ELISA test to detect antigens in the blood, with specificity of 100% (no cross-reactivity occurs with *C. vulpis*) and sensitivity of 98%. This test gives better results than Baermann`s test. Schnyder et al. (2011) have also synthesized a very sensitive sandwich ELISA test to determine circulating antigens, which is highly sensitive (97.5%) and specific (94%). The authors are convinced that these tests are eligible for the diagnosis, monitoring and screening for diseases caused by the nematode *A. vasorum*. The PCR technique is of significance also, although there are still no commercialized tests (Al-Sabi et al., 2010).

Given the fact that angiostrongylosis in dogs is increasingly frequent and has increasing clinical and epidemiological characteristics, highly sensitive and specific diagnostic methods are necessary in order to set up as precise a diagnosis as possible. Better techniques are also needed for epizootic investigations. Real time quantitative PCR provides an opportunity for a much more efficient method of diagnosing *A. vasorum* in dogs, with higher threshold sensitivity than traditional diagnostic tests. If combined with other complementary methods such as ELISA, it could be of significant epizootic and clinical use, and may be of importance for the development of a model for controlling this disease (Jefferies et al., 2009). Identification of affected dogs can be even more precise if the bronchoalveolar lavage is examined with PCR (Canone et al., 2016).

Aelurostrongylosis diagnostics

Suspecting this disease is based on clinical symptoms and radiography. Clinical signs are right heart insufficiency with haemoglobinuria and possible cardio-respiratory collapse, and the presence of miliary or nodular shadows in the lungs (Stepanović et al., 2015). However, accurate and reliable diagnosis is made by identifying the parasite during the post-mortem, using the post-mortem lung parenchyma method "clutch preparation", or finding developmental stages of larvae in samples of faeces collected on three consecutive days (Traversa et Guglielmini, 2008). Given that adult females of *A. abstrusus* lay their eggs in the bronchial tree of diseased animals, coprological examination makes it possible to diagnose L1 larvae.

Coprological examination

Coprological examination methods used are: direct smear of faeces, native Pataki check, and the Baermann`s method. Direct smear methods are inexpensive and simple to perform, but they are insensitive given the small amount of sample and can detect only a high intensity infection (animals infected with a large number of parasites that

excrete large numbers of larvae in the faeces) (Traversa and Guglielmini, 2008; Humm and Adamantos, 2010).

The flotation method is not sensitive enough, considering the amount of sample (5-10 g) which is reviewed, and damage to larvae can occur under the influence of flotation solution. The solutions which are used for carrying out the flotation method (saline or concentrated sugar) have a high specific gravity and the high osmotic pressure can lead to dehydration of the larvae and modification of their appearance. This greatly complicates their identification, especially for inexperienced diagnosticians (Traversa and Guglielmini, 2008; Traversa et al., 2010).

It has been shown that a saturated aqueous solution of zinc sulphate (specific gravity of 1.18 to 1.2) is the most reliable flotation solution for the identification of L1 larvae. Practical experience has shown that flotation solutions can result in 40-90% of the cases producing a negative result, even in animals which are positive for aelurostrongylosis (Conboy, 2004; Traversa et al., 2010).

Baermann`s method is the gold standard for diagnosing aelurostrongylosis, considering that L1 larvae of *A. abstrusus* express a positive hydro/thermal tropism (Traversa and Guglielmini, 2008; Conboy, 2009). An accurate morphological and morphometrical description is needed of the larvae in L1 form, for them to be correctly identified. Identification of larvae in the faeces is based on size (about 360 μm in length) and morphological characteristics - part of the tail in the shape of the letter "C" and the presence of subterminal spines (Lalošević et al., 2001; Traversa et al., 2010).

However, Baermann`s method has its limitations. It requires a long time (12-48 hours), there may be false-negative results during the prepatent period, and the cyclical secretion of larvae, which is characteristic of Angiostrongylidae, should be taken into account. For this reason, faecal samples need to be collected over three consecutive days, in order to increase the sensitivity of the test (Guglielmini and Traversa, 2008; Conboy, 2009). Larvae also can be diagnosed from the trans-tracheal aspirate or bronchoalveolar lavage - BAL (Lalošević et al., 2001; Ribiero and Lima, 2001; Ribiero et al., 2014).

Ribiero et al. (2014) demonstrated that cellular BAL fluid (BALF) evaluation provides useful information to the veterinary clinician, especially when larvae of *A. abstrusus* are found, enabling the illness to be differentiated from other pulmonary feline disease. The BALF allows us to retrieve cells and L1 larvae, which provides additional information about the inflammatory process caused by aelurostrongylosis.

Molecular techniques

Molecular diagnostic methods have eliminated the constraints that exist with conventional methods for diagnosing the aelurostrongylosis. Lately, as a common laboratory technique, PCR has been used with increasing frequency to detect genetic markers in the parasite ribosomal DNA. This method can be used for the diagnosis of faecal samples and pharyngeal swabs, with a specificity of 100% and a sensitivity

of 97%, which indicates that it is much more reliable than the conventional method (Traversa et al., 2008).

In cats with subclinical respiratory syndrome infections, the nematode *A. abstrusus* was determined (Lalošević et al., 2001), which is important in the differential diagnosis of respiratory diseases of cats. In addition to other lung infections caused by parasites (*Eucoleus aerophilus* and *Paragonimus kellicotti*) in cats, bacterial, viral and fungal infections, allergies and nasopharyngeal polyps must be excluded (Mandic, 2015). In addition to other differences, *P. kellicotti* eggs have a cap at one pole (Ellis et al., 2010), and the eggs of *E. aerophilus* have asymmetrically placed poles with caps and a shell streaked with densely spaced furrows linked by a large number of connections (Ilić et al., 2015); based on these differences, they can be differentiated from the eggs of *Aelurostrongylus* spp. (Traversa et al., 2009; Ellis et al., 2010).

During differential diagnosis of aelurostrongylosis, one should pay attention to the presence of larvae from a lesser-known parasite (*Oslerus rostratus*), because they look similar, but they can be differentiated with Baermann`s method in samples of cat faeces (Conboy, 2009). However, prolonged presence of lesions in the lungs, after ejection of parasites in feces, greatly complicates the diagnosis (Lautenslaugther, 1976). For this reason, it is necessary to take into account tuberculosis, tumors and mycosis during differential diagnosis (Hawkins, 1995).

In addition to *A. abstrusus*, it was recently found that *Troglostrongylus brevior* (Crenosomatidae) can also be the cause of pulmonary diseases in cats. These two parasites are biologically similar, occupy the same ecological niche and can simultaneously cause disease in cats, but are difficult to distinguish during differential diagnosis, due to the morphological similarity of their L1 stages.

There is a molecular technique (duplex PCR), for simultaneous detection and differential diagnostic distinction of *T. brevior* and *A. abst*rusus. In this technique, the individual L1 larvae of both pathogens are isolated from a representative sample of faeces, in order to execute their morphological identification. Duplex PCR proved to be efficient and highly sensitive for the simultaneous detection of the two pulmonary nematodes and could find application in both molecular epizootic studies as well as in testing the efficiency of the treatment and control of these diseases (Annoscia et al., 2014).

CRENOSOMOSIS DIAGNOSTICS

The most effective and the best method for determining the type of infection caused by *C. vulpis* is testing samples of faeces with Baermann`s method; in practice, this is a centrifugal flotation in a saturated aqueous solution of zinc sulfate (Cobb and Fisher, 1992; Peterson et al., 1993).

Coprological examination

The listed procedures are successful in revealing L1 larvae, a diagnostic stage of this nematode. L1 larvae are not always detectable by standard methods, which are mainly used in veterinary clinics, but they can be isolated with tracheal lavage examination of suspicious animals (Shaw et al., 1996).

Direct smear of faeces and the flotation method are not sufficiently reliable methods, since the concentrated solutions of sugar and salt can lead to differences in osmotic pressure, which can cause the dehydration of the larvae. As a result of such reactions and the changed look of the larvae, it is difficult or impossible to correctly identify them. Baermann`s method is the most accurate, since live L1 larvae of *C. vulpis* exert a positive hydro/thermal tropism (Traversa and Guglielmini, 2008; Conboy, 2009). If larvae are present in the faeces, a complete morphometric and morphological identification must be made, where it is necessary to distinguish each individual pulmonary nematode larva, leeches and larvae of nonparasitic nematodes or nematodes from plants, which can be inadvertently sampled in the field together with the faeces (Conboy, 2009).

L1 larvae of *C. vulpis* can be identified based on morphological appearance and size (length 246-308µm) (Craig and Anderson, 1972). Evaluation of morphological characteristics of the larvae is done by adding drops of Lugol`s iodine solution on the edge of the cover plate, which ensures fixation (immobilization) and dyeing of the larvae. Eggs of *C. vulpis* reconstituted from tracheal lavage have thin walls, measuring 72x43 µm and contain fully developed L1. Examination of faeces by flotation (with aqueous $ZnSO_4$ specific gravity 1.18) should be performed to exclude infection caused by the *Oslerus (Filaroides) osleri* and *Capillaria aerophila* (Shaw et al., 1996).

For parasitic infections in dogs, differential diagnosis is needed to exclude *Dirofilaria immitis* and *A. vasorum* (Ilić et al., 2015), as well as infectious diseases, nasopharyngeal polyps, allergic bronchitis, the presence of foreign bodies and tumors (Traversa et al., 2010).

Molecular techniques

Molecular methods for diagnosing infections caused by *C. vulpis* are not yet sufficiently developed, but continuous molecular and genetic research is underway, aimed at finding the mitochondrial ribosomal genetic markers of this nematode.

Identification of genetic markers for *C. vulpis* is necessary for its morphological identification, and this should enable some progress in terms of molecular diagnostics. Finding three (out of four) 12S rDNA haplotypes in one red fox from Calabria indicates the existence of significant variability of genetic material in the population of the nematode host (Tolnai et al., 2015).

For domestic and wild carnivores in Italy, molecular typing of the nematode *C. vulpis* was performed by sequencing mitochondrial (12S ribosomal DNA (rDNA)) and nuclear (18S rDNA) ribosomal genes. Four haplotypes were identified using the

12S rDNA gene target, among which is the most common (78.5%), haplotype I. There was no significant genetic variability in 18S rDNA. Molecular identification was in line with a clear separation of specific subtypes identified and phylogenetic analysis of mitochondrial and ribosomal genes (Latrofa et al., 2015).

Avise points out that the genetic variability in the same kind of parasite can be explained by crossing different animal hosts, or by the different geographical landscapes of the same host species, or a high percentage of mutations in the mitochondrial genetic material (Avise, 1994). This assumption is supported by the fact that the a higher rate of nucleotide variability between haplotypes II and III (0.9%) was identified in *C. vulpis* isolated from red foxes that came from geographical regions which were located close to each other (Calabria and Campania). The high prevalence of 12S rDNA haplotype I (78.5%) in red fox *C. vulpis* points to the recent spread of this parasite in this animal population, which is derived from the knowledge that this one haplotype was isolated from a dog and a skunk. This theory is supported by the 18S rDNA sequences of all the isolates being identical to those isolated from foxes (Chilton et al., 2006). All this points to the fact that foxes play a most important role in the spread of this nematode (Tolnai et al., 2015).

Given that there are currently insufficient data available, it is still not possible to determine precisely which exact haplotypes are present in Europe or in the United States (Latrofa et al., 2015).

CONCLUSION

Given the increased prevalence of angiostongylosis in dogs in neighboring countries, it is of great importance to improve the diagnostic methods for this condition, which will contribute to the control of this disease, given that treatment at an early stage is very simple and successful. *A. abstrusus* is the most common lung parasite in free-living cats and can have a very large clinical pathological significance, particularly in cases of high intensity infections. Therefore, in cats exhibiting signs of bronchopneumonia, causes of parasitic aetiology should be suspected. Crenosomosis in dogs can pose a serious health problem in veterinary medicine, as indirect and/or direct contact with the fox population, or stray dogs and cats, can be a potential source of infection in urban areas, increasing the risk of transmission of these zoonotic parasites. Small practice clinicians should include this nematode in their differential diagnosis, especially for those dogs living in rural areas where red foxes could appear.

For the timely implementation of effective treatment of cardiopulmonary metastrongyloidosis, accurate diagnosis is necessary, and is the most important for the prognostics of the infected carnivore.

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Authors' contributions

MM participated in the design of the sudy, assisted in data collection, analysis and translation in English. IT has designed the paper, selected references for the presentation and wrote manuscript. SP; has made substantial contribution to the conception an design, acquisition and interpretation of data, drafted the manuscript and prepared final version for publication. OS participated in the design of the sudy, assisted in data collection and analysis. DS has made critical revise of the concept, has given substantial contribution to analysis and interpretation, and been involved in drafting the manuscript and revising critically. All authors read and approved the final manuscript, and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy and integrity of any part of the work are appropiately.

Competing interests

The authors declare that they have no competing interests.

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KARDIOPULMONARNA METASTRONGILIODOZA PASA I MAČAKA DOPRINOS ZA POSTAVLJANJE DIJAGNOZE

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Kratak sadržaj

Uvod. U poslednjih petnaest godina, na evropskom kontinentu i širom sveta značajno se povećala prevalencija kardiopulmonalnih metastrongilidoza kod pasa i mačaka, naročito onih uzročnika koji imaju najveći značaj za veterinarsku praksu (*Angiostrongylus vasorum*, *Aelurostrongylus abstrusus* i *Crenosoma vulpis*).

Cilj i pristup. Cilj ovog rada je da se detaljnim kliničko-parazitološkim osvrtom istakne značaj ove grupe helmintoza i da se prikažu najnovija saznanja vezana za mogućnosti njihove dijagnostike kod pasa i mačaka.

Ključni nalazi i zaključak. Efekti globalnog zagrevanja, pomeranje granica habitacije vektora, učestalo kretanje i transport životinja u druga epizootiološka područja, intenziviran promet robe i velika fluktuacija ljudi, samo su neki od potencijalnih faktora koji bi mogli uticati na ovakvu dinamiku pojavljivanja, održavanja i širenja kardiopulmonalnih nematodoza kod mesojeda. Za blagovremeno sprovođenje efikasnog tretmana obolelih jedinki neophodna je precizna dijagnostika ovih parazitoza, što u krajnjem ishodu značajno može uticati na prognostički tok oboljenja kod inficiranih mesojeda. S obzirom na stepen njihove patogenosti, poteškoće koje se javljaju u postavljanju dijagnoze, kao i činjenicu da neke od njih predstavljaju potencijalnu opasnost po zdravlje ljudi, navedena oboljenja imaju izuzetan klinički i epizootiološki značaj.

Ključne reči: angiostrongiloza, elurostrongiloza, krenozomoza, pas, mačka, dijagnostika